Abstract

Increased release of free fatty acids (FFA) from visceral fat cells to the portal venous system may cause several metabolic disturbances in obesity. However, this hypothesis and the underlying mechanism remain to be demonstrated. In this study catecholamine-induced lipid mobilization through lipolysis in omental adipose tissue was investigated in vitro in 25 markedly obese subjects (body mass index range 35–56 kg/m²) undergoing weight reduction surgery and in 19 nonobese subjects (body mass index range 20–28 kg/m²) undergoing cholecystectomy. Release of FFA and glycerol, induced by norepinephrine or adrenergic receptor subtype-specific agonists, were determined in isolated omental fat cells. The obese subjects had higher fat cell volume, blood pressure, plasma insulin levels, blood glucose, plasma triglycerides, and plasma cholesterol than the controls. There was evidence of upper-body fat distribution in the obese group. The rate of FFA and glycerol response to norepinephrine was increased twofold in the cells of obese subjects; no significant reutilization of FFA during catecholamine-induced lipolysis was observed in any of the groups (glycerol/FFA ratio near 1:3). There were no differences in the lipolytic sensitivity to β₁ or β₂-adrenoceptor specific agonists between the two groups. However, β₂-adrenoceptor sensitivity was ~ 50 times enhanced (P = 0.0001), and the coupling efficiency of these receptors was increased from 37 to 56% (P = 0.01) in obesity. Furthermore, the obese subjects demonstrated a sixfold lower α₂a-adrenoceptor sensitivity (P = 0.04). β₂-Adrenoceptor sensitivity, but not α₂a, β₁, or β₂-adrenoceptor sensitivity, correlated with norepinephrine-induced lipolysis (r = 0.67, P = 0.0001) and fat cell volume (r = 0.71, P = 0.0001).

In conclusion, catecholamine-induced rate of FFA mobilization from omental fat cells is accelerated due to elevated rate of lipolysis in obesity, mainly because of an increased β₂-adrenoceptor function, but partly also because of a decreased α₂a-adrenoceptor function. This promotes an increased release of FFA to the portal system, which may contribute to the parallel metabolic disturbances observed in upper-body obesity. (J. Clin. Invest. 1995. 95:1109–1116.)

Key words: adrenoceptors • β₂-adrenoceptor • fat cells • free fatty acids • lipolysis

Introduction

It has long been established that obesity is associated with increased morbidity and mortality. The distribution of body fat is also of importance for the complications to obesity as reviewed (1–3), upper-body obesity has a stronger association to metabolic and cardiovascular diseases than lower-body obesity. A plausible trigger for the complications in obesity may be free fatty acids (FFA) produced by the visceral fat depot through lipolysis in fat cells in this region, as discussed in detail elsewhere (2, 3). The visceral fat mass is increased in upper-body obesity (4). Only this fat depot has direct access to the liver through the portal system. The release of excess FFA into the portal circulation may have a number of unwanted effects on the liver, such as glucose intolerance, impaired metabolism and action of insulin, and altered lipoprotein synthesis as reviewed (2, 3, 5, 6). The resulting insulin resistance may, in turn, contribute to the development of diabetes and hypertension and cause a further deterioration in lipoprotein metabolism.

Nevertheless, an increase in FFA production from the visceral fat depot in obesity remains to be established, and the possible underlying mechanisms have not been determined. However, in vivo data suggest that lipolysis regulation is altered in obesity, a finding which is less apparent in lower-body obesity than in upper-body obesity (7–9). In this respect, it is of interest to study catecholamine-induced FFA and glycerol release from visceral fat cells since, in adult humans, catecholamines are the only hormones with marked acute lipolytic effects (10). The catecholamines effects are modulated through four adrenoceptor subtypes, i.e., stimulation via β₁, β₂, and β₃ adrenoceptors and inhibition via α₂-adrenoceptors (11). The β₂-adrenoceptor has recently been cloned (12) and its involvement in the adipocyte lipolysis of laboratory animals is well documented (13). Although β₂-adrenoceptors are expressed in several human fat depots (14), they have little lipolytic action on the subcutaneous fat tissue but a marked lipolytic function in fat cells from the visceral region (15–18).

At present it is not possible to study the β₂-adrenoceptor with classical techniques such as radioligand binding and protein determination, in contrast to the other adrenoceptor subtypes involved in lipolysis regulation. However, one can gain insight into the function of all adrenoceptor subtypes by investigating the lipolysis concentration–response relationship for selective adrenergic agonists, as reviewed elsewhere (19). The half-maximum effective concentration (ED₅₀) can be determined and reflects specific agonist–adrenoceptor interactions, since at this concentration the selective agonist either has no or insignificant interactions with other adrenoceptor subtypes.

We have presently investigated the adrenergic regulation of
lipolysis in visceral fat cells from the omental region by performing concentration-response experiments with norepinephrine and various synthetic adrenergic subtype specific agonists, determining the end products in lipolysis (glycerol and FFA) in massively obese and matched nonobese subjects. The obese subjects were selected for a high waist-to-hip ratio to, above all, study subjects with an enlarged visceral fat mass. An increased rate of FFA mobilization after catecholamine stimulation was observed in omental fat cells of obese subjects. This is attributed mainly to a marked increase in the lipolytic function of β1-adrenoceptors.

Methods

Patients. The study comprised 25 upper-body obese, otherwise healthy, drug-free subjects (body mass index 43±1 kg/m², range 35–56) undergoing weight reduction surgical treatment through open or laparoscopic procedures, and 19 nonobese subjects (body mass index 24±1 kg/m², mean±SE, range 20–28) undergoing elective laparoscopic cholecystectomy at Huddinge University Hospital. All subjects were Caucasians. The two groups were matched for sex, age, and smoking habits and were clinically characterized before surgery. In both groups about a third were men. The ages of the subjects ranged from 19 to 59 yr. The waist-to-hip ratio, the sagittal diameter, and the systolic and diastolic blood pressures were measured in the supine position on the day before surgery. All obese women had a waist-to-hip ratio > 0.95 and all obese men had a waist-to-hip ratio > 1.00. The amount of visceral fat was calculated from the sagittal trunk diameter (D) by a computer tomography-calibrated equation (0.731 × D − 11.5 for men and 0.370 × D − 4.85 for women), as described previously in detail by Sjöström (4). The sagittal diameter was obtained by measuring the distance from the examination table to a horizontal crossbar placed over the abdomen of a recumbent subject at the crista level. The blood pressure was measured with a mercury sphygmomanometer on the right arm. The systolic and diastolic pressures were determined by using phases I and V of the Korotkoff sounds, the values are the means of three consecutive measurements after a 10-min rest. After an overnight fast, the study subjects rested in bed for 15 min, thereafter venous blood samples were obtained for determinations of metabolic laboratory parameters. These were later analyzed by the hospital’s routine chemistry laboratory, except for insulin, which we measured with a radioimmunoassay kit (Pharmacia, Uppsala, Sweden).

General anesthesia was induced at 8 a.m. by a short-acting barbiturate and maintained by fentanyl and a mixture of oxygen and nitrous oxide. Intravenous saline was administered before the fat biopsies, which were taken from the major omentum at the beginning of the operation. The study was approved by the Ethics Committee of Karolinska Institute, Stockholm, and all the patients gave informed consent to participate in the study.

Isolation of fat cells and determinations of cell size and number. For technical reasons, it was not possible to obtain specimens larger than 0.3–1.0 g of omental fat during the laparoscopic operations, also the weight reduction surgery was initiated with laparoscopic technique in about half of the cases. The adipose tissue was immediately transported to the laboratory in saline at 37°C, and isolated fat cells were prepared from the fat specimens by collagenase treatment, as described by Rodbell (20). The cells were kept in an albumin solution, as described below, and the cell density of the fat cell suspension was kept constant by slow stirring with the aid of a magnet. Direct microscopic determination of the fat cell diameter, performed according to the method of Di Girolamo and co-workers (21), was calculated by using 200 cells from each subject. The mean fat cell volume and weight were determined, taking into account the skewness in the distribution of the cell diameter and using the method described by Hirsch and Gallian (22). The total lipid content in each incubation was determined gravimetrically after organic extraction. Assuming that lipids constitute > 95% of the fat cell weight, the number of fat cells can be calculated by dividing the total lipid weight by the mean cell weight. This indirect method for determining the fat cell number was compared with a tedious direct method (23), where all cells are counted in appropriately diluted cell suspensions. The two methods gave almost identical results in 10 consecutive experiments (r = 0.97), using linear regression analysis.

Lipolysis experiments. The lipolysis assay has been described previously in detail (24). Briefly, 0.2 ml of diluted suspensions of isolated fat cells (5,000–10,000 cells/ml) were incubated in duplicate for 2 h with or without increasing concentrations of either the natural catecholamine norepinephrine, the nonselective β-adrenergic agonist isoprenaline, the selective β1-adrenoceptor agonist dobutamine, the selective β2-adrenoceptor agonist terbutamine, the selective partial β3-adrenoceptor agonist CGP 12177 (18), or the selective α1-adrenoceptor agonist UK 14304 (25). All incubations were performed at 37°C in Krebs-Henseleit phosphate buffer (pH 7.4), supplemented with glucose (1 g/liter), bovine serum albumin (20 g/liter), and ascorbic acid (0.1 g/liter), with air as the gas phase. The ligidans were added simultaneously at the start of the incubation. The concentration range used for each drug depended on its lipolytic performance. The concentration was ranged from 10⁻¹² to 10⁻⁴ mol/liter. The same batches of collagenase and albumin and the same stock solutions of adrenoreceptor agonists were used throughout the study. As discussed in detail previously (26), adenosine leaking out from isolated fat cells may interfere with the α1-adrenoceptor-mediated antilipolytic effect of catecholamines. In our dilute incubation system there is minimal influence of adenosine contamination. However, in the α1-adrenoceptor experiments, adenosine deaminase (1 mU/ml) was added to prevent antilipolytic interactions with traces of adenosine that might still be present and might induce additional inhibitory effects (26) in the diluted cell suspensions. The influence of adenosine on lipolysis in a fat cell system like ours is, on the other hand, negligible when adipocytes are stimulated with lipolytic drugs, as discussed previously (27). Therefore, we preferred not to add adenosine deaminase in the remaining concentration-response experiments. In the experiments with UK 14304, the incubation medium was also supplemented with 10⁻⁷ mol/liter 8-bromo cyclic AMP to increase the initial (basal) rate of lipolysis, which was too low in visceral fat cells to be inhibited by UK 14304. We have shown previously that 8-bromo cyclic AMP-induced lipolysis can be fully inhibited by antilipolytic agents in human fat cells (28). There was no difference in sensitivity to 8-bromo cyclic AMP between obese and nonobese subjects. The glycerol concentration after the 2-h incubation was determined in a cell-free aliquot by a bioluminescence method (29).

All agonists caused a concentration-dependent stimulation or inhibition of glycerol release that reached a plateau at the highest agonist concentrations. Concentration-response curves for glycerol release were used to determine the concentrations of the adrenoceptor agonists, giving half of their own maximum stimulation (B) or inhibition (α1a). These ED₅₀ values (expressed as log mol/liter) were determined by linear regression analysis of log-logit transformation of the ascending (β-agonsists) or descending (α₁-agonsists) part of the individual concentration–response curves (30). This mathematical method for determining ED₅₀ gave the same results as visual fitting of concentration–response curves on lin-log paper. Lipolysis rates in the absence of, or in the presence of, maximum effective agonist concentrations were related to fat cell number. The maximum lipolytic effects indicate agonist responsiveness. The isoprenaline experiments were used to determine the intrinsic activities of the selective lipolytic agents. This drug is always a full agonist since it fully activates all β-receptor subtypes at maximum effective isoprenaline concentrations (10, 11). By comparing the maximum effects of selective β-adrenoceptor agonists with isoprenaline, one can evaluate the coupling efficiency of the selective agonist to the effector system (19). Intrinsic activities were determined as the percentage of the maximal isoprenaline response for each experiment separately. Since the amount of fat tissue available was limited, we were not able to perform experiments in all cases. We decided on the following priorities: (a) the selective β1-adrenoceptor agonists (n = 44); (b) norepinephrine (n = 38); and (c) α1a-adrenoceptor ago-
the fat

Fat-to-hip ratios

glycerol production

and distribution

insulin resistance.

words, the levels, but pressure,
in respect, respectively,

of purity

oxidase.

Lilly sulfate

hydrochloride

obtained from

ascorbate oxidase,

acyl-CoA oxidase

cells were exactly

in 22 out of 44

FFA analyses.

unpaired statistical comparisons.

Likewise,

subgroups.

The effects of the twofold higher

fat cell FFA response and glycerol

response to norepinephrine than the

nonobese subjects, both when basal

values were included (repeated ANOVA,

P = 0.01 and P = 0.006, respectively)
or subtracted (repeated ANOVA,

P = 0.03 and P = 0.02, respectively).
The ratio of glycerol release to FFA release was almost 1:3 in these experiments as

seen in Fig. 1. The individual ratios were calculated on net lipolysis (minus basal) at 10^{-7} mol/liter of norepinephrine,

when there was a maximum rate of FFA release in both groups.
The glycerol/FFA ratio was 1:2.86±0.55 in the obese and 1:2.66±0.24 in the nonobese subjects. These values did not differ statistically.

The increased effect of catecholamines on glycerol and FFA in the obese subjects can be attributed to a change in any of the stimulatory \( \beta_1 \), \( \beta_2 \), and \( \beta_3 \)-adrenoceptor agonists or the inhibitory alpha-adrenoceptors. To investigate these four receptors, fat
cells were incubated with selective \( \beta_1 \) and \( \beta_2 \)-adrenoceptor agonists, i.e., dobutamine (\( \beta_1 \)), terbutaline (\( \beta_2 \)), CGP 12177 (\( \beta_3 \)), or UK 14304 (alpha\(_2\)) and glycerol release was determined.
The mean concentration–response curves are shown in Fig. 2 as a percentage of their maximum effect, to elucidate differences in agonist sensitivity between groups (i.e., left- or rightward shift in the concentration–response curve). There were no apparent differences in \( \beta_1 \) or \( \beta_2 \)-adrenoceptor sensitivity between the two groups. However, the obese subjects were clearly much more sensitive to the \( \beta_3 \)-adrenoceptor agonist CGP 12177. Furthermore, the obese group demonstrated a somewhat lower alpha\(_2\)-adrenoceptor sensitivity. The mean ED\(_{50}\) values (log mol/liter) for stimulation or inhibition, with the different agonists, as determined from the individual dose–response curves, are given in Table II. The ED\(_{50}\) values for CGP 12177 and UK 14304 were significantly different between the groups. \( \beta_3 \)-Adrenoceptor sensitivity was 50 times increased (\( P = 0.0001 \)), and alpha\(_2\)-adrenoceptor sensitivity was 6 times de-

<p>| Table I. Clinical Data in Obese and Nonobese Subjects |</p>
<table>
<thead>
<tr>
<th>Age (yr)</th>
<th>Obese</th>
<th>Nonobese</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (male/female)</td>
<td>9/16</td>
<td>7/12</td>
<td>NS</td>
</tr>
<tr>
<td>Body mass index (kg/m(^2))</td>
<td>43±1</td>
<td>24±1</td>
<td></td>
</tr>
<tr>
<td>Waist-to-hip ratio (men)</td>
<td>1.06±0.01</td>
<td>0.96±0.02</td>
<td>0.001</td>
</tr>
<tr>
<td>Waist-to-hip ratio (women)</td>
<td>1.00±0.01</td>
<td>0.90±0.02</td>
<td>0.0001</td>
</tr>
<tr>
<td>Visceral fat vol (liters)</td>
<td>8.59±0.64</td>
<td>2.77±0.38</td>
<td>0.0001</td>
</tr>
<tr>
<td>Fat cell volume (pl)</td>
<td>620±37</td>
<td>262±27</td>
<td>0.0001</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>142±3</td>
<td>125±3</td>
<td>0.0002</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>89±2</td>
<td>76±1</td>
<td>0.0001</td>
</tr>
<tr>
<td>Plasma insulin (mU/liter)</td>
<td>17.4±1.5</td>
<td>8.1±0.9</td>
<td>0.0001</td>
</tr>
<tr>
<td>Plasma glucose (mmol/liter)</td>
<td>5.64±0.13</td>
<td>5.15±0.09</td>
<td>0.007</td>
</tr>
<tr>
<td>Plasma triglycerides (mmol/liter)</td>
<td>1.92±0.13</td>
<td>1.48±0.14</td>
<td>0.02</td>
</tr>
<tr>
<td>Total cholesterol (mmol/liter)</td>
<td>5.91±0.17</td>
<td>5.24±0.22</td>
<td>0.02</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/liter)</td>
<td>1.12±0.07</td>
<td>1.32±0.09</td>
<td>NS</td>
</tr>
<tr>
<td>Plasma norepinephrine (nmol/liter)</td>
<td>1.91±0.12</td>
<td>1.79±0.09</td>
<td>NS</td>
</tr>
<tr>
<td>Plasma epinephrine (nmol/liter)</td>
<td>0.15±0.01</td>
<td>0.16±0.03</td>
<td>NS</td>
</tr>
</tbody>
</table>

The values are means±SE. The groups were compared using the Student’s t test. Visceral fat cell number was obtained by dividing the total visceral fat volume (liter) by the visceral fat cell volume (pl).
increased \( (P = 0.04) \) in obesity. In addition, the sensitivity to norepinephrine was also significantly enhanced by five times in the obese group (Table II).

The intrinsic activity, i.e., the maximum effect of a selective agent in relation to the maximum effect of isoprenaline, was also determined for the selective \( \beta \)-adrenoceptor agonists. The mean values for the maximal lipolytic response induced by isoprenaline were 16.4±1.3 and 15.3±1.9 in the obese and nonobese group, respectively. The difference was not significant. Dobutamine and terbutaline were both full agonists relative to isoprenaline in the visceral fat of both obese and nonobese subjects in this study, while CGP 12177 was a partial agonist. However, the intrinsic activity of CGP 12177 was significantly higher in the obese subjects \( (56±5 \text{ vs } 37±5\%, \ P = 0.01) \). The absolute concentration–response curves (\( \mu \text{mol} \text{glycerol}/10^7 \text{ cells}/2 \text{ h} \)) for the three \( \beta \)-adrenoceptor agonists are given in Fig. 3. The lipolytic response to CGP 12177 was clearly more pronounced in the obese subjects than in the nonobese subjects (repeated ANOVA, \( P = 0.01 \)). On the other hand, no significant differences were seen in regard to the dobutamine or terbutaline responses. Neither did the maximum \( \alpha_2 \)-adrenoceptor–mediated antilipolytic response induced by UK 14304 differ between obese and nonobese subjects. It was defined as glycerol release in the absence of UK 14304 minus glycerol release in the presence of the maximum effective concentration of UK 14304. The responses to UK 14304 were 5.9±1.5 and 5.7±1.0 \( \mu \text{mol} \text{glycerol}/10^7 \text{ cells}/2 \text{ h} \) in the obese and the nonobese subjects, respectively.

Finally, the importance of adrenoceptor subtype sensitivity for the variations in lipolytic function of catecholamines was investigated in the whole study material. The relationship between \( \beta_1\), \( \beta_2 \), and \( \beta_3 \)- and \( \alpha_2 \)-adrenoceptor agonist sensitivity \( (\log ED_{50} \text{ for dobutamine, terbutaline, and CGP 12177) on the one hand, and norepinephrine-induced lipolysis, on the other hand, was first investigated by single regression analysis. } \beta_3\text{-Adrenoceptor sensitivity correlated with all concentrations of norepinephrine-induced lipolysis, with } r \text{ values ranging from 0.46 to 0.67 and } P \text{ values ranging from 0.0017 to 0.0001. The relationship between } \beta_3\text{-adrenoceptor sensitivity and lipolysis induced by 1 nmol/liter of norepinephrine } (r = -0.67, \ P = 0.0001) \text{ is given in Fig. 4. Neither the } \beta_1\text{ nor the } \beta_2\text{ nor the } \alpha_2\text{-adrenoceptor sensitivities correlated significantly with norepinephrine-induced lipolysis. The } r \text{ values for } \beta_1\text{ and } \beta_2\text{-adrenoceptors ranged from 0.10 to 0.36 and 0.01 to 0.34, respectively, while the } r \text{ values for the } \alpha_2\text{-adrenoceptor ranged from 0.01 to 0.30. By a stepwise regression analysis, the relative contribution of } \beta_1\text{, } \beta_2\text{, or } \beta_3\text{-adrenoceptor sensitivity was compared with norepinephrine-induced lipolysis. The adrenoceptor with the highest partial correlation coefficient was entered as the first step and the lowest F value (i.e., variance ratio) for entry was set to 4. } \beta_3\text{-Adrenoceptor sensitivity was entered as the first and only step (adjusted } r^2 = 0.44, \ F = 29.9).
We also investigated the possible correlation between fat cell volume and \( \beta \)-adrenoceptor sensitivity (i.e., \( \text{ED}_{50} \)) using linear regression analysis (figure not shown). There was a strong association between fat cell volume and \( \beta_1 \)-adrenoceptor sensitivity \((r = -0.71, \ p = 0.0001)\). However, no significant relationship was observed between fat cell volume and the sensitivities of the \( \beta_2 \) and the \( \beta_3 \)-adrenoceptor \((r = -0.08 \text{ and } 0.16, \text{ respectively})\).

### Table II. Lipolytic Sensitivity to Adrenergic Agonists in Obese and Nonobese Subjects

<table>
<thead>
<tr>
<th>( \text{ED}_{50} ) nonobese ( \log \text{ mol/liter} )</th>
<th>( n )</th>
<th>( \text{ED}_{50} ) obese ( \log \text{ mol/liter} )</th>
<th>( n )</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Norepinephrine (glycerol)</td>
<td>(-8.11 \pm 0.12)</td>
<td>18</td>
<td>(-8.76 \pm 0.14)</td>
<td>20</td>
</tr>
<tr>
<td>Dobutamine (( \beta_1 ))</td>
<td>(-8.43 \pm 0.24)</td>
<td>19</td>
<td>(-8.75 \pm 0.21)</td>
<td>25</td>
</tr>
<tr>
<td>Terbutaline (( \beta_2 ))</td>
<td>(-7.36 \pm 0.18)</td>
<td>19</td>
<td>(-7.46 \pm 0.18)</td>
<td>25</td>
</tr>
<tr>
<td>CGP 12177 (( \beta_3 ))</td>
<td>(-7.11 \pm 0.20)</td>
<td>19</td>
<td>(-8.81 \pm 0.20)</td>
<td>25</td>
</tr>
<tr>
<td>UK 14304 (alpha2)</td>
<td>(-9.40 \pm 0.21)</td>
<td>14</td>
<td>(-8.69 \pm 0.27)</td>
<td>10</td>
</tr>
</tbody>
</table>

The values are mean±SE. \( \text{ED}_{50} \), the concentration that induces 50% of the maximal lipolytic or antilipolytic response.

### Discussion

This demonstrates for the first time that the rate of FFA mobilization from the visceral fat cells is increased in obesity, at least when investigated in vitro in omental fat cells from massively obese subjects. Norepinephrine stimulation of visceral fat cells in obese subjects resulted in a simultaneous doubling of the average FFA and glycerol responses, compared to nonobese subjects. Furthermore, glycerol to FFA ratio was near 1:3 in both groups, which indicates a minimal level of reesterification of FFA during lipolysis. This clearly indicates that the increased catecholamine-induced FFA mobilization in obesity is due mainly to an altered lipolytic response and to a lesser extent secondary to an alteration in the reutilization of FFA.

The present effects of norepinephrine on FFA and glycerol release in obese subjects are to some extent in opposition with previous reports of in vivo studies showing decreased lipolytic responses to catecholamine infusions in obese as compared to lean subjects \( (7, 32, 33) \). However, in these studies the overall lipolysis rate was measured in vivo. This mainly reflects the lipolytic effects on the subcutaneous fat mass which, due to its high proportion of the total fat mass (\( \sim 80\% \)), dominates the response. It has recently been demonstrated in vitro that the lipolytic sensitivity to epinephrine and norepinephrine is impaired in the subcutaneous region in upper-body obese subjects \( (34, 35) \) indicating regional differences in the impact of abdomi-
Figure 3. Rate of lipolysis induced by selective β-adrenergic agonists. Values for glycerol release (means±SE) are derived from the experiments depicted in Fig. 2. The responses were expressed as micromoles of glycerol/10^7 cells/2 h. Omental fat cells from obese (filled boxes) and nonobese (open boxes) subjects were incubated with increasing concentrations of selective adrenoceptor agonists, i.e., dobutamine (β1) (A), terbutaline (β2) (B), and CGP 12177 (β3) (C).

Fig. 4. Relationship between β3-adrenoceptor sensitivity and catecholamine-induced lipolysis. Scatterplot showing the linear relationship between lipolytic sensitivity (ED50) for CGP 12177 and the glycerol response to a physiological norepinephrine concentration (1 nmol/liter) in the entire study group (n = 38). r = -0.67, P = 0.0001.
sis was not possible to investigate as no methods to quantify the \( \beta_1 \) receptor protein or the mRNA in human fat are available at present. There are yet no radioligands suitable for this purpose because of methodological problems with nonspecific binding, as reported previously (15, 18). Furthermore, the low level of \( \beta_1 \)-adrenoceptor mRNA expression makes quantitative methods based on both Northern blot or RNase protection unsuitable because their detection limit is too high (38). It has been possible to perform a quantification of the \( \beta_1 \)-adrenoceptor mRNA signal in fat cells with the polymerase chain reaction technique in cells with a high expression level (39), but it is not useful when the expression level is low. At present, there are no antibody techniques available to measure the amount of human \( \beta_1 \)-adrenoceptor protein. A recently constructed specific human \( \beta_1 \)-adrenergic antibody is, however, not suitable for a quantitative assay (Hausemann, M., personal communication).

The findings with \( \beta_1 \)-adrenergic subtypes are representative for the whole material because the three selective \( \beta_1 \)-adrenoceptor agonists were used in all subjects. Furthermore, a sixfold decrease in alpha\(_2\)-adrenoceptor sensitivity was observed in the obese subjects’ omental fat cells. However, this finding was based on a limited number of subjects, and the alpha\(_2\)-adrenoceptor was not the major focus in the present investigation. This receptor has complex interactions (located at the level of the so-called G-proteins) with the \( \beta_1 \)-adrenoceptors in human fat cells, which can be investigated in detail only in very large amounts of tissue (26). However, it is thought likely that a decrease in the alpha\(_2\)-adrenoceptor function contributes to the increase in lipolytic action of catecholamines in omental fat cells in obesity.

The increased \( \beta_1 \)-adrenoceptor function and decreased alpha\(_2\)-adrenoceptor function in the obese subjects were highly unexpected findings. It has been reported that the \( \beta_1 \)-adrenoceptor mRNA levels in white fat of obese Zucker rats was reduced by \( \sim 70\% \), as compared with lean control animals (40). Furthermore, it has been shown that the alpha\(_2\)-adrenoceptor agonist sensitivity is higher in obese than in lean dogs (41). These data indicate that animal models are not always valid for conclusions concerning the mechanisms underlying obesity in humans.

The clinical relevance of the present findings should also be considered. None of the obese patients included in this study had any overt disease apart from obesity. However, these patients showed as a group several signs of classical complications, which together form the so-called insulin resistance or metabolic syndrome. The results of anthropometric measurements strongly indicated an upper-body type of fat distribution, although such measures may be less accurate in massively obese subjects as discussed in detail recently (42). Furthermore, the obese subjects had hyperinsulinemia, glucose intolerance, dyslipidemia, and elevated blood pressure, which are all factors resulting in an increased risk for cardiovascular disease (1–3, 5, 6). We suggest that these abnormalities may, to some extent, be induced by an increased FFA release from the visceral fat depots in upper-body obesity secondary to an increased \( \beta_1 \)-adrenoceptor function in the omental fat cells. Whether these conclusions are true also for moderately obese individuals remains to be investigated.

Our data concerning the \( \beta_1 \)-adrenoceptor may also be useful for future pharmacotherapeutic research in the fields of obesity and metabolic diseases. Until now, pharmacological \( \beta_1 \)-adrenoceptor research has focused on the development of potential \( \beta_1 \)-adrenergic agonists that may activate brown fat cells and possibly result in thermogenic effects (13, 43). Our data indicate that it could be of an equally high interest to develop \( \beta_1 \)-adrenoceptor antagonists that would inhibit the metabolic effects of a \( \beta_1 \)-adrenoceptor—induced increase in the FFA release from visceral white fat tissue in certain obese subjects.

We presently used very diluted fat cell suspensions to study lipolysis. There are several advantages to this method. A large number of experiments can be performed on a small amount of tissue. The problems with adenosine contamination in the medium are minimized. The cells appear to be very sensitive to physiological stimulation since a clear lipolytic effect is obtained with norepinephrine concentrations in the same range as those in the circulation (1–10 nmol/liter).

In conclusion, we show for the first time that the FFA mobilization from visceral (i.e., omental) fat cells is increased in obesity. This increment is due to an enhanced lipolytic action of catecholamines in omental fat cells, which in turn is secondary to a markedly increased \( \beta_1 \)-adrenoceptor action and, to a minor extent, to a slightly decreased alpha\(_2\)-adrenoceptor action in the adipocytes. These alterations may increase the rate of FFA mobilization to the portal system during lipolysis and contribute to the metabolic disturbances in obesity.

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References


\( \beta_1 \)-Adrenoceptors in Obesity

1115


